The effects of perinatal morphine-exposure on rat dams and their offspring

Ph.D. Thesis

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1. INTRODUCTION

1.1 Human backgrounds: epidemiology and clinical aspects

According to 2009 annual report of the European Monitoring Centre for Drugs and Drug Addiction, (EMCDDA) 1.2-1.5 million European (of 15-64 years old) can be regarded as problematic opioid user. In Europe, abuse of heroin is henceforward responsible for the majority of drug related morbidity and mortality (that is 75% of all the deaths due to substance abuse). Exact Hungarian epidemiological data are not available; however, the incidence of the opioid dependence is estimated c.a. 0.32‰ by a Hungarian treatment guideline.

Between 1996 and 1998 in the USA 2.8% of pregnant women abused any substances according to a representative survey. Textbooks assume the incidence of opioid abuse from 1-2% to 21% in different pregnant populations.

According to the report of the Hungarian National Focal Point of EMCDDA pregnancy was diagnosed in 3% of the all cases of drugabusers of a Hungarian basic social care (Józan Babák Klub, Club of Sane Babies).

The pregnancy could be unrecognised a good while since opioid dependent women may identify early signs of pregnancy as withdrawal symptoms. Maternal opioid abuse or social/living conditions can be life threatening directly for both the mother and the developing foetus. Complications of delivery are common, withdrawal symptoms appear in more than 79% and 53% of the mature and premature neonates of dependent mothers, respectively. Symptoms are diverse and not all of them emerge: neurological symptoms: irritability, constant, harsh crying, more pronounced Moro-reflex, muscle hypertonia, tremor, myoclonus. Convulsion appears in 7.8% and 1.2% of the neonates of methadone and heroin abuser mothers, respectively. Gasrtointestinal symptoms: diarrhoea, vomiting, hyperphagia or feeding disorders are common. Respiratory
symptoms as tachypnoea, hyperpnoea and several autonomic system disorders may appear: sneezing, hiccup, increased lacrimation, hyperpyrexia. Superficial excoriations can be seen on the baby’s skin. Nowadays mortality of the neonatal withdrawal syndrome has decreased under 1%. Long term consequences of perinatal opioid exposure for the child could hardly be assessed since behaviour and development are strongly influenced by environmental factors, as well. Decreased growth, attention deficit and hyperactivity, learning difficulties were described. Some behavioural disorders were reported in children adopted by families with good socio-economic status.

1.2 Endogenous opioids in gestation, lactation and postpartum behaviour, data from animal studies
Endogenous opioids play an important role in maintenance of pregnancy as triggers of parturition and maternal behaviour. In general, endogenous opioids inhibit stress-response during gestation. Dynorphin and enkephalins inhibit oxytocin-release in the posterior hypophysis. In late pregnancy this inhibitory effect decreases, oxytocin can be released, inducing delivery and its optimal procession and facilitating immediate maternal care. Until the end of gestation tubero-infundibular dopaminergic neurons are inhibited by opioids, too. Upregulated opioid mechanisms decrease after labour back to the nonpregnant level.

1.3 Effects of opioid agonists and antagonists on the postpartum behaviour
Opioids inhibit maternal behaviour. This inhibitory effect can be antagonised by opioid antagonists. Various parameters of maternal behaviour can be studied in rats: pup retrieval into the nest, placentophagia, pup caring, lactation positions, quality of the nest. In the early postpartum period morphine impairs maternal behaviour after continuously increased dosing, as well. Sensitisation was measured to the maternal behavioural inhibitory effect of morphine after its constant, repeated administration. Maternal behavioural impairing effect of morphine was reported after the withdrawal of maternal morphine-treatment. Maternal behaviour was dose-dependently inhibited by µ-agonists (DAGO, morphine, β-endorphin). DAGO, a selective µ-agonist was shown more potent than morphine and β-endorphin. Maternal behaviour was unaffected by the δ-agonist DPDPE and the κ-agonist U50488H.

1.4 Opioid dependence in animal studies
1.4.1 Physical dependence
Characteristic withdrawal symptoms appear in opioid-dependent rat when opioid-treatment is stopped: „wet dog shakes”, irritability, vocalisation, diarrhoea. In pregnant dependent dams besides maternal withdrawal symptoms significant foetal hyperactivity can be identified due to foetal opioid withdrawal. Naloxon precipitates withdrawal symptoms in both neonate and five days old pup of dams exposed to chronic morphine treatment.

1.4.2 „Psychic” withdrawal
After withdrawal of chronic opioid-treatment anxiety-like behaviour on elevated plus maze test (rats spend less time in the open arms of the maze) and hypolocomotion of adult rats can be assessed as „psychic” withdrawal symptoms. If withdrawal is precipitated with opioid-antagonists (naloxone, naltrindol), hypolocomotion become more pronounced. In contrast to adult animals, in pups exposed pre- or postnatally to morphine locomotor activity is increased after precipitation of withdrawal by an antagonist. Conditioned place aversion paradigm is generally used for detecting withdrawal symptoms of drugs of abuse (negative reinforcement effect). In
morphine dependent rats place aversion could be evoked by naloxone in a significantly lower dose than to precipitate physical withdrawal.

1.4.3 Positive reinforcement
Besides self-administration conditioned place preference test is generally used to detect abuse potential of a substance. After animal was conditioned with a drug with positive reinforcement effect (e.g. β-endorphine, amphetamine, phencyclidine, morphine, heroin, cocaine, diazepam, Δ9-tetrahydrocannabinol), the drug-paired compartment of the place preference equipment is preferred even by the untreated (naive) animal. This effect of certain drugs is dose dependent (e.g. morphine, heroin, amphetamine) and can be inhibited by the antagonist of the substance. Place preference can be measured for a long time after withdrawal of morphine. In adult male rats exposed prenatally to morphine the positive reinforcement effect of morphine and cocaine further increases in self administration tests, saccharine-preference also increases and place preference is more pronounced.

1.5 Antinociceptive effect of morphine
Tolerance develops after continously administered µ-receptor agonist opioids including morphine to their antinociceptive effect. This phenomenon was observed in pups exposed perinatally to methadone or buprenorphine: antinociceptive effect of morphine was decreased in four or twenty-one days old pups versus control.

2 OBJECTIVE
In the literature few data can be found on the effects of constant, moderate dose (10 mg/kg s.c.) morphine administration. This treatment regimen was applied in my work continously during gestation and lactation (perinatal period) and its effects were studied on dams and its pups:

In dams:
1. How does morphine-treatment influence maternal behaviour?
2. Do the effects of morphine and its antagonist naloxone change on maternal behaviour after morphine-treatment?
3. Does measurable dependence develop (physical or psychic) in dams?
4. Does the antinociceptive effect of morphine change (can tolerance or sensitisation be measured)?

In offspring:
1. Could behavioural changes be detected in pups exposed perinatally to constant morphine? Is there any difference between whole perinatal and prenatal (in utero) or postnatal (during lactation) exposure?
2. Does measurable dependence develop (physical or psychic) in offspring exposed to perinatal morphine?
3. Does perinatal morphine exposure increase the abuse liability in adulthood (vulnerability)?
4. Does the antinociceptive effect of morphine change due to the perinatal morphine exposure? Is there any difference between whole perinatal and prenatal (in utero) or postnatal (during lactation) exposure?

3 METHODS
3.1 Experimental animals and treatment groups
Nullipara female Wistar rats weighing 200-220 g were mated and treated once daily with 10 mg/kg s.c. morphine from the first day of gestation until the separation (21st postpartum day) or the given day of experiment. Therefore treatment groups of dams were SAL-group or MO-group. In U/1 offspring experiments the effects of the whole perinatal (during the whole gestation and lactation) morphine exposure were studied (control s/s-offspring and perinatally exposed m/m-ones were compared). In U/2 experiments the effects of
prenatal -in utero- (m/s-group) and postnatal -exposure after birth-(s/m-group) morphine-exposure were investigated.

### 3.2 Materials

morphine-hydrochloride, naloxone, methylendioxy-metamphetamine (MDMA, ecstasy)

### 3.3 Experiments

#### 3.3.1 Experiments in dams only

**Maternal behaviour** was studied in the early postpartum period in dams only. Different parameters of spontaneous behaviour were observed and scored: observed maternal behaviour: active grooming (active nursing or cleaning of pups) passive nursing, littering or manipulating on nest shaves, observed non-maternal behaviour: out-of-the-nest behaviour: eating, drinking, self-grooming and other behaviours, like rearing, ambulation, resting and sleeping. **Retrieval test** was also performed: all of the pups were removed from the maternal cage, five minutes later five of them were replaced to the corner opposite of the nest. Latency to retrieve the first and the fifth pups to the nest was measured. Spontaneous changes of maternal behaviour in the early postpartum period, duration of one dose of morphine (10 mg/kg s.c.), dose-effect relationship and the effects of naloxone (3 mg/kg s.c.) on maternal behaviour and morphine-disrupted maternal behaviour were investigated.

#### 3.3.2 Experiments in offspring only

**Spontaneous locomotor activity** was studied in young adolescent (23 days old) and late adolescent (42 days old) pups using „Conducta” motimeter. Horizontal and vertical movement of the animal was registered by infrared diodes. Exploratory activity (open field behaviour) and adaptation to a novel environment (habitation) were recorded.

#### 3.3.3 Experiments both in dams and offspring

Dependence was measured after precipitation of withdrawal symptoms by naloxone both in dams and pups shortly after separation. According to Buckett’s method the following **physical withdrawal symptoms** were scored: writhing (3 points), vocalisation (squeeling) (2 points), diarrhoea (2 points), teeth chattering (1 point), ptosis (1 point), wet dog shake (1 point). Behavioural signs of „psychic” withdrawal were studied on elevated plus maze and in „Conducta” motimeter. **Conditioned place aversion** was performed using the same device as in conditioned place preference test. When the animal can freely move between the dark and light compartments of the place preference device the difference in times spent in the dark compartment after and before conditioning with naloxone refers to the level of place aversion. Place aversion experiment was performed in the adult offspring. **Conditioned place preference** was performed in dams after conditioning with 3 mg/kg morphine. It was also studied in adult offspring after conditioning with 3 mg/kg morphine and 1 mg/kg MDMA. During these experiments illicit drugs were administered in the light compartment. The antinociceptive effect of morphine was studied by irradiant tail-flick method in dams after separation and in young adolescent and adult offspring. Besides these tests, body weights of both dams and pups were measured daily.

### 4 RESULTS

#### 4.1 Dams

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<th>4.1.1 Body weight</th>
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<td>The body weights of morphine-treated dams change similarly to that of the controls, there was no difference between two treatment groups.</td>
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4.1.2 Maternal behaviour
Between 2nd and 9th postpartum days maternal behaviour did not change significantly as postpartum time passed. Morphine strongly disrupted maternal behaviour 30 minutes after administration. The effect was smaller but still significant 2 hours after the administration, 4 hours after the administration it was insignificant and undetectable 24 hours after the administration. Higher doses of morphine resulted in greater inhibition of maternal behaviour in both treatment groups. In the MO-group the inhibitory effect was quantitatively higher, furthermore, a lower dose of morphine (3 mg/kg) decreased maternal behaviour significantly only in the MO-group and not in control animals. This phenomenon may be due to sensitisation. Naloxone disrupted maternal behaviour in the MO-group and not in the control one. Naloxone antagonised the maternal behavioural impairing effect of morphine only in the control group while the antagonism was incomplete in the MO-group.

4.1.3 Dependence
In dams only very mild physical withdrawal symptoms could be precipitated by high dose naloxone (10 mg/kg s.c.): mean score was 1.86 out of maximal 10. „Psychic” withdrawal signs were observed neither on locomotor test nor on elevated plus maze. On the contrary, naloxone induced significantly more pronounced place aversion in the MO-group than in the control one.

4.1.4 Positive reinforcing effect of morphine
Morphine induced significantly stronger place preference in the MO-group.

4.1.5 Antinociceptive effect of morphine
Antinociceptive effect of morphine was unchanged in the MO-group, tolerance could not be measured.

4.2 Offspring
4.2.1 Body weight
Pups exposed prenatally to morphine were born with lower body weight than their control peers. If the pups were further exposed to morphine by maternal milk, their body weight reached and later overtook that of the controls. When the maternal morphine-treatment started only at birth (s/m-group) or was withdrawn at birth (m/s-group), weight gain of pups was slower than that of the controls.

4.2.2 Spontaneous locomotor activity
In experiment U/1 two days after separation there was no difference between m/m- and control-groups in exploratory (open field-) behaviour, while adaptation of m/m-offspring and control ones to a novel environment (habituation) was similar. The decreased habituation was more pronounced in males than in females. At late adolescence this difference in habituation was not detectable. In experiment U/1 two days after separation there was no difference between s/m- and m/s-groups in exploratory behaviour. Adaptation to a novel environment was significantly decreased in male m/s-pups compared to s/m- and control offspring.

4.2.3 Dependence
Mild physical withdrawal could be measured in pups exposed with morphine in the whole perinatal period (m/m-group). In males and females the mean score was 1.28 and 0.28, respectively (out of maximal 10) as scored by Buckett’s method. „Psychic” withdrawal signs were not detectable in m/m-offspring on locomotor test shortly after separation; in contrast, naloxone induced more pronounced place aversion in the adult m/m-offspring.
4.2.4 Positive reinforcing effect of illicit drugs
In experiment U/1 morphine in doses of both 1 and 3 mg/kg s.c. induced significantly more pronounced place preference in adult offspring exposed to morphine in the whole perinatal period than in control ones. This effect of morphine was stronger in females than in males. Comparing the effects of pre- and postnatal morphine-exposure the effect of prenatal morphine exposure was shown to be significantly more marked.

1 mg/kg s.c. MDMA induced significantly more pronounced place preference in offspring exposed to perinatal morphine. Gender difference was not found.

4.2.5 Antinociceptive effect of morphine
The antinociceptive effect of morphine was decreased in perinatally morphine-exposed (m/m-) males on the third day after separation. Antinociceptive tolerance was not detectable in adult m/m- males. Tolerance was not detectable in either female m/m-group or in offspring of U/2 experiment either after separation or in adulthood.

5 CONCLUSIONS
The most important conclusions of present work are:
Maternal behaviour is disrupted in dams treated with constant, moderate dose morphine in the perinatal period, furthermore, sensitisation develops to the maternal behavioural inhibitory effect of morphine. Besides, sensitivity of dams changes to the opioid agonists/antagonists.
Constant, moderate dose perinatal morphine decreases the adaptation of male offspring to a novel environment as well as the sensitivity to the antinociceptive effect of morphine in the early adolescence.

Decreased habituation is more pronounced after prenatal exposition than after postnatal exposition.
Constant, moderate dose morphine strongly increases the positive reinforcing effects of both morphine and MDMA. This effect of morphine is more pronounced in females than in males. Furthermore, it is also detectable when the exposition happened only in utero; suggesting that increased risk of dependence in adulthood may be due to fetal neurological effects.
According to the results in both dams and offspring it can be concluded that increased vulnerability to the positive reinforcing effect of illicit drugs is independent from dependence after exposition/treatment. Dependence was very weak after our treatment regimen.

Although human drug abuse can be hardly translated to animal models our results show that our treatment regimen (generally not reported in the literature) -i.e. constant, relatively small dose of morphine administered once daily during the gestation and lactation-results in several fine behavioural changes in dams and its offspring. In offspring exposed to morphine both short and long term harmful behavioural effects appear that may be due to the direct pharmacological effects of morphine and decreased maternal care. In my opinion, it is very important that vulnerability to abuse of drugs is increased even in adult offspring and may concern both “maternally mediated” morphine and other substances as well. Abuse liability will be increased in general by perinatal MO-exposure.
6 LIST OF THE AUTHOR’S PUBLICATION

6.1 Papers related to doctoral thesis:


6.2 Papers not closely related to doctoral thesis:

